Agricultural Materials as Renewable Resources

Nonfood and Industrial Applications

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Biopolymers from Fermentation

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Biopolymers are used for a variety of food and nonfood applications, and many can be produced by microbial fermentation of agricultural commodities. Commonly used fermentation medium components include corn starch, corn gluten meal, and corn steep liquor. Commercialized bacterial exopolysaccharides produced by fermentation include dextran, xanthan, gellan, cellulose and curdlan. Bacterial polyhydroxyalkanoates recently have found use in the manufacture of biodegradable plastics. New, potential products obtained by microbial fermentation include yeast glucans and several fungal and bacterial exopolysaccharides. We initiated a screening program in our laboratory, resulting in the identification of several high-yielding alginate-producing bacterial strains, as well as several producers of novel exopolysaccharides. Bacterial alginates may substitute for algal alginates in certain applications. The complete structures of most of the novel bacterial exopolysaccharides were determined.

Hydrocolloids or water-soluble gums are used as thickeners, stabilizers, emulsifiers and gelling agents in food and nonfood industries. Plant hydrocolloids have been employed for such purposes for thousands of years (1). However, it was not until the 1960s that the first microbial hydrocolloid, the bacterial exopolysaccharide (EPS) xanthan gum, was available for commercial use. Bacterial EPSs are found outside of the cell either in the form of a tightly-held capsule or a loosely-held slime layer. They are usually anionic and of high molecular weight. Xanthan gum is produced by the bacterium *Xanthomonas campestris,* causal agent of black rot disease of cruciferous crops. It was first isolated by Lilly and coworkers (2) in the late 1950s and then extensively studied for possible commercial applications at the USDA, ARS, Northern Regional Research Center (now the National Center for Agricultural Utilization Research, NCAUR) located in Peoria, IL. This polysaccharide was approved by the U.S. Food and Drug Administration (FDA) for general food use in 1969 and first sold by the Kelco Company (now a division of Monsanto Chemical Company). Xanthan

gum is an acidic, high molecular weight heteropolysaccharide with a pentasaccharide repeating unit consisting of a cellulosic backbone with trisaccharide side chains on alternate glucose residues (3). The side chains are substituted with pyruvate and acetate. Due to its unique physical properties xanthan is used as a thickener, stabilizer and suspending agent for several food and nonfood applications (4). Xanthan forms highly viscous aqueous solutions at low concentrations, stable over a wide range of pH, temperature and salt concentrations. In addition, these solutions exhibit high pseudoplasticity. Most recently, xanthan is being employed as a shortening replacer for food use. Annually, about 20,000 metric tons of xanthan gum are used for commercial purposes in the U.S.A. (5).

Due at least in part to high development costs, it would be twenty-four years (in 1993) before another microbial EPS, gellan gum, was approved by the FDA for general food use in the U.S.A. Gellan gum is produced by the saprophytic bacterium *Pseudomonas (Auromonas) elodea*. It consists of a repeating unit of an acidic, linear tetrasaccharide containing the sugars glucose, glucuronic acid and rhamnose, as well as acetate and glycerate (6). The deacylated form produces a firm, brittle gel in the presence of monovalent or divalent cations (4). The polysaccharide was sold beginning in 1990 as an agar substitute, especially useful for plant tissue culture applications.

Curdlan is a water-insoluble, linear β-(1,3)-linked D-glucan produced as an EPS by *Alcaligenes faecalis* var. *myxogenes* (7). This polymer forms gels when heated to 55 C or above and subsequently cooled. Curdlan also gels when alkaline solutions are neutralized. In Japan curdlan is sold by Takeda Chemical Ind., Ltd. for food use and by Wako Pure Chemical Ind., Ltd. for nonfood use. Certain modified curdlans are reported to have anticancer activity (7). At the present time, curdlan is not approved for food use in the U.S.A.

A bacterial EPS that has many nonfood applications is dextran (8). Commercial dextran is obtained primarily from *Leuconostoc mesenteroides* strain NRRL B-512 or its derivative B-512(F). This strain was isolated at the NCAUR in the 1940s (9). The polysaccharide produced by this strain consists solely of α-(1,6)-linked glucan with variable degrees of branching to the O-3 of the backbone moieties (8). The polymer is synthesized extracellularly from sucrose by the action of the enzyme dextransucrase. It is possible to obtain dextrans of narrow molecular weight ranges for specific applications by controlling culture conditions. For example, a 70 kD dextran is used as a blood expander and a 40 kD dextran is used in organ perfusion solutions. Modified dextran is also used for the manufacture of a variety of chromatographic media (8). Annual consumption of dextran for nonfood uses in the U.S.A. is approximately 2000 metric tons (5). Currently, dextran is not approved for food use in the U.S.A.

A fifth bacterial EPS, cellulose, has found food and nonfood commercial applications. Cellulose is a linear, non-water soluble polymer composed solely of β-(1,4)-linked glucose. The source of bacterial cellulose for commercial use is *Acetobacter xylinum*. Bacterial cellulose, in contrast to plant cellulose, can be obtained in highly purified form free of lignin. This material has a high degree of crystallinity, a much larger surface area than plant cellulose, and has excellent mechanical strength and absorptive capacity (10). Bacterial cellulose is used to prepare a dessert called "nata" in the Philippines (11), and is also used by Sony

Corporation to manufacture high quality audio speaker systems. Several other potential applications are under study (10).

Additional bacterial EPSs such as emulsan and alternan and fungal polymers such as pullulan, scleroglucan and yeast glucans (12,13) are currently either not produced commercially or are produced in small amounts. These polymers are being studied for a variety of potential commercial uses.

Intracellular bacterial polymers have several potential as well as realized commercial uses. Much recent industrial and academic interest has centered around using bacterial polyesters for the production of biodegradable plastics. Under conditions of adequate carbon availability and nutrient stress (14) many bacteria synthesize polyhydroxyalkanoic acids (PHAs) as intracellular energy reserve materials, stored as inclusion bodies. The PHAs consist of hydroxyalkanoic acids with chain lengths between 3 and 14 carbon atoms. The specific PHAs produced are dependent on the bacterium and the carbon source available in the fermentation broth. The bacterium *Alcaligenes eutrophus* has been the most studied PHA producer. Zeneca BioProducts utilizes this bacterium to produce a biodegradable resin (BioPol) made from polyhydroxybutyrate- hydroxyvalerate copolymer. The resin is used to make biodegradable films, coatings and containers.

All commercially available bacterial EPSs are currently produced by batch fermentation using complex media. Agricultural byproducts commonly used as carbon and energy sources for fermentation include glycerol, molasses, corn starch and starch hydrolysates, glucose, sucrose and whey (65% lactose). Commonly used nitrogen sources include corn steep liquor, corn gluten meal, dried distillers solubles, yeast extract, fish meal, soybean meal and cottonseed flour (5,15). Several fermentation variables affect polymer yield and quality (e.g., molecular weight, degree of substitution with non-sugar moieties, level of various contaminating macromolecules). These include the carbon source, nitrogen source, the carbon to nitrogen ratio, the levels of various cations and anions, available oxygen levels, temperature and stage of microbial growth at time of harvest. Polymer molecular weight can be indirectly affected by the effects of culture conditions on EPS depolymerases. In general, the nature of the EPS produced is independent of carbon source, but there are exceptions (5).

Acidic Exopolysaccharides of Group I Pseudomonads

Alginates. The term alginate encompasses a group of structurally related, linear, acidic polysaccharides containing varying amounts of O-acetylated β-1,4-linked D-mannuronic acid and its C-5 epimer L-guluronic acid (16). These two uronic acids are present as homopolymeric or heteropolymeric block structures. The number of homopolymeric blocks of guluronate in the polymer determines its gelling properties. A high amount of polyguluronate leads to the formation of firm, brittle gels in the presence of divalent cations, particularly calcium, while low polyguluronate alginates form more elastic gels (16). Alginates comprise a major structural polymer in brown algae, and these algae, harvested from the ocean, are currently the sole source of alginates for commercial use. Algal alginates have many applications in food and nonfood industries as thickeners and gelling agents. They were first produced commercially in California in 1929. Annual worldwide consumption of alginates is

estimated at approximately 23,000 metric tons (5). The algal species, age at harvest, geographic location of harvest and plant part extracted all can have a significant effect on both the level of guluronic acid present and the cost of alginate (17). The production of alginate by bacterial fermentation of agricultural commodities would allow for a product with a constant composition and availability.

Several years ago we initiated a study of EPSs produced by the rRNA homology group I pseudomonads (18). This group of bacteria consists primarily of pseudomonads which produce fluorescent iron-binding pigments (siderophores) when grown on media low in iron. Members of this important group of bacteria include human pathogens, saprophytes, and plant pathogens. They are the subject of intensive study due to their pathogenic potential, their extreme metabolic capabilities useful for bioremediation applications and their potential as biocontrol agents for a variety of serious plant diseases. The type species of this group is the opportunistic human and plant pathogen *P. aeruginosa.* At the time the project was initiated, very little was known about the EPSs produced by this group of pseudomonads. *Pseudomonas aeruginosa* was previously shown to produce alginate (19), and alginate-producing variants had been obtained by laboratory manipulations of *P. fluorescens, P. putida* and *P. mendocina* (20,21). Alginates produced by pseudomonads were shown to be similar to algal alginates except for two important properties. First, there were no homopolymeric blocks of guluronic acid present, which affects their gelling properties in the presence of cations and, secondly, some mannuronic acid residues were mono and/or disubstituted with acetate (13).

We began our studies using the soybean pathogen *P. syringae pv. glycinea.* Several strains of the bacterium were demonstrated to produce alginate in glucose-containing broth media and in infected plant leaves (22). The alginates were acetylated and contained up to 20% guluronic acid. The guluronic acid content of the alginates produced by individual strains was dependent on the particular environmental conditions at the time of synthesis. This differed from alginates produced by *P. aeruginosa,* whose mannuronic acid to guluronic acid ratio appeared to be independent of growth conditions for each particular strain (23). Early studies in our laboratory utilizing ^{1}H-NMR and enzymatic assays using a guluronate-specific alginate lyase indicated the possible presence of low levels of homopolymeric guluronic acid block structures in some *Pseudomonas* alginates. However, further studies by others indicated that pseudomonad alginates are devoid of such sequences (5,16). Results of high-performance size exclusion chromatography indicated that the alginates produced in infected plants were higher in molecular weight (2.0×10^4 to 4.7×10^4 D) than corresponding alginates produced *in vitro.* The relatively low molecular weights (3.8×10^3 to 5.2×10^3 D) of the *in vitro* produced alginates were most likely due to the action of alginases synthesized by the producing bacteria (Fett, unpublished). Subsequently, we found that alginates were produced by additional plant pathogenic and saprophytic group I pseudomonads, but not by pseudomonads which did not belong to group I (24,25,26).

The bacterium *P. aeruginosa* is not a viable source of alginates due its pathogenicity towards humans. The other well-studied alginate-producing bacterium, *Azotobacter vinelandii,* is also not used currently because it has a high oxidation rate (27). Thus, we conducted further investigations to determine whether any of the other group I pseudomonads included in our studies might be useful for the production of

alginates (28). A total of 115 strains of fluorescent *Pseudomonas* species (*P. cichorii, P. fluorescens, P. syringae* and *P. viridiflava)* were tested for yields of alginates when grown in batch culture in a proprietary liquid medium (PLM) (Kelco). The PLM contained either fructose or glucose (both at 5%, w/v) as the primary carbon and energy source. Selected strains were also grown in a modified Vogel and Bonner medium (MVBM) containing gluconate (5%, w/v). This medium was previously formulated by others to support maximal alginate production by the human pathogen *P. aeruginosa* (29). Cultures were incubated for five days at 24 C with shaking (250 to 300 r.p.m.). The yields of alginate present in isopropanol-precipitated material were estimated by use of a colorimetric assay for uronic acid content (30). Maximal yields of alginates were 5 g/L for PLM with fructose, 3 g/L for PLM with glucose and 9 g/L for MVBM. Culture fluids containing such high amounts of alginate were highly viscous to gel-like. Starch was not tested as a carbon and energy source since none of the pseudomonads tested could utilize this feedstock.

The yields of alginates obtained represent a good starting point for further development of selected *Pseudomonas* strains for commercial production of bacterial alginates. Bacterial alginates could be used for applications which do not require the formation of strong gels, e.g., as viscosifiers for textile dyes. A knowledge of the environmental factors affecting alginate synthesis would be useful to develop fermentation conditions which would allow for increased yields. To this end, we determined the effect of various stresses and other culture parameters on the yields of alginates produced by representative group I pseudomonads other than *P. aeruginosa*. Results indicated that many fluorescent pseudomonads respond to various environmental stresses by increasing production of alginates (31). Hyperosmotic conditions brought on by addition of sodium chloride (0.2 to 0.5 M) to a complex liquid medium led to a significant increase in alginate production; up to 8-fold on a mg alginate/L culture media basis and up to a 22-fold increase on a mg alginate/cell dry weight basis. Similarly, addition of ethanol (1 to 3%) to the medium caused up to a 12-fold increase in alginate production on a mg alginate/L culture media basis. However, strains which did not produce alginate in control cultures did not produce alginates under stress conditions. The results were very similar to those found for *P. aeruginosa* (32). The data indicated that environmental stress can increase constitutive alginate synthesis, but by itself cannot induce alginate production. A very recent report, however, demonstrated that inclusion of copper into a solid medium induced alginate production by several copper-resistant strains of *P. syringae* (33). We noted that constitutive alginate production was quite low (a maximum of 49 mg alginate/L) (31) so that even a 22-fold increase did not represent an acceptable commercial yield. Addition of sodium chloride or ethanol to culture media which supported high yields of alginates (3 to 9 g/L) did not lead to further increases. The primary limiting factor in these cultures may have been oxygen availability due to the highly viscous nature of the culture fluids. The effect of various nitrogen sources (ammonium sulfate, potassium nitrate, sodium nitrate and sodium glutamate) and divalent cations on alginate production by a strain of the plant pathogen *P. viridiflava* strain 671m cultured in a completely defined medium also was determined. Ammonium sulfate supported the greatest production of alginate on a mg/g cell dry weight basis. Inclusion of Mn^{+2} (1 mM) and Mg^{2} (10 mM) in the growth medium

greatly stimulated alginate production, while inclusion of Fe^{+2} was inhibitory (Singh, et al., unpublished). Similar results have been reported for *P. aeruginosa* (29,32,34).

How environmental signals are sensed by alginate-producing bacteria and how the message is transduced to the cytoplasm has been the subject of extensive molecular studies on *P. aeruginosa* and additional group I pseudomonads. The regulation of alginate production is extremely complex, with both global and specific two-component signal-transducing regulatory systems interacting with each other. Primary regulation of alginate biosynthesis by pseudomonads is by specific activation of promoters of two alginate biosynthetic genes, *algC* and algD. A number of positive and negative regulatory genes have been identified (32,35,36,37). A recently described regulatory gene, designated *repA*, was cloned from *P. viridiflava* (38). RepA is a global regulator, controlling not only alginate synthesis, but also synthesis of extracellular enzymes required by *P. viridiflava* to attack its host plants.

Novel exopolysaccharides. Based on our early experiments with group I pseudomonads, we hypothesized that all of these bacteria produce alginate as an acidic EPS, but further studies proved this hypothesis to be incorrect. The group I pseudomonads turned out to be a rich source of novel acidic EPS structures. As a species, *Pseudomonas marginalis*, a pectolytic plant pathogen which causes soft-rot of harvested fruits and vegetables, produces three different acidic EPSs (39,40,41). Their acidity comes from pyruvate, succinate or lactate substituents, not from uronic acid as usually found for acidic bacterial EPSs. The first was a galactoglucan which we named marginalan (type A, Fig. 1). At the time of publication the exact location of the succinate substituent was not known. Recent data indicate that the galactose moiety is disubstituted with succinate and pyruvate whereas glucose is not substituted (42). Subsequently, we found marginalan to be produced by certain strains of *P. fluorescens* and *P. putida* (25,26). Two additional acidic EPSs are produced by strains of *P. marginalis* (types B and C, Fig. 1), but so far their production appears to be specific to a very limited number of strains of this species (40,41). A fourth novel acidic EPS is produced by the type strain (ATCC 17588) of the group I, nonfluorescent, opportunistic human pathogen *P. stutzeri* (Fig. 1) (43). Again, this polymer is acidic solely due to the presence of a lactic acid substituent. Genetically-manipulated *P. stutzeri* was reported previously to produce alginate as an acidic EPS (44). As a species, *P. stutzeri* is quite diverse (18) so it is not surprising that more than one acidic EPS is produced. An acidic EPS other than alginate is also produced by the mushroom pathogen *P.* "gingeri" (Fig. 1) (45). This was the first EPS of the group I pseudomonads, other than alginate, shown to contain uronic acid. It is of interest that the *P.* "gingeri" EPS is identical in structure to the EPS produced by *Escherichia coli* strain K55 (46) and differs only in the location of the acetate group from *Klebsiella* type 5 capsular EPS (47).

Most recently, we have been studying acidic EPSs produced by three additional group I pseudomonads: saprophytic *P fluorescens* strain H13 (26), saprophytic *P. chlororaphis* strain B2075 and *P. flavescens* strain B62, a recently described walnut pathogen (48). The composition of the *P. fluorescens* strain H13 polymer is unusual for the group I pseudomonads as it contains glucosamine (26). The extracellular material produced by *P. chlororaphis* is a mixture of a hexosamine-containing polymer and alginate. The EPS produced by *P. flavescens* strain B62 also appears to

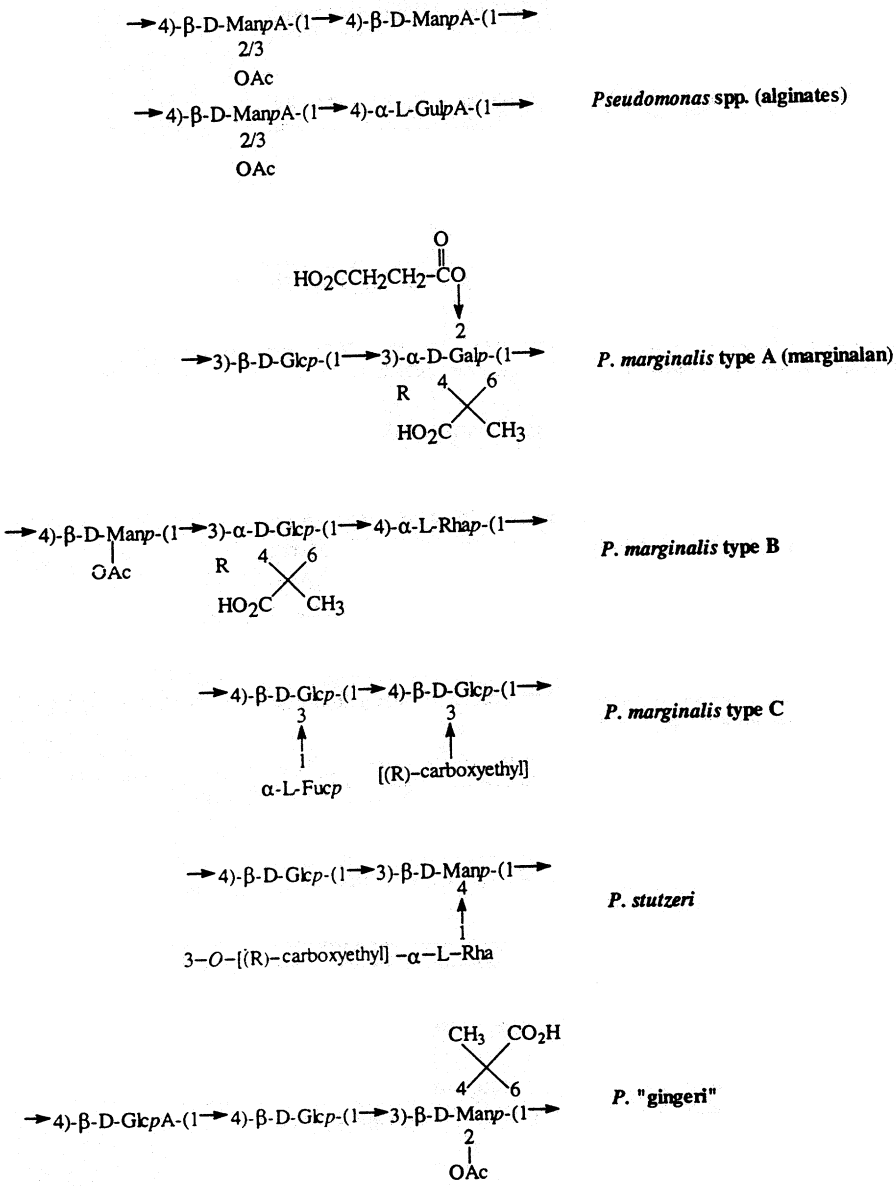

Figure 1.

Structures of exopolysaccharides produced by group I pseudomonads. ManA, mannuronic acid; GulA, guluronic acid; OAc, O-acetyl group; Glc, glucose; Gal, galactose; Man, mannose; Rha, rhamnose; Fuc, fucose; GlcA, glucuronic acid; *p*, pyranose form of the sugar.

be unusual for this group as it contains a uronic acid substituted with lactic acid (Cescutti *et al.*, unpublished).

Physical properties. In order to determine if any of the EPSs isolated from the group I pseudomonads might have commercial applications, a variety of physical measurements as well as utility tests were run. All EPS samples examined in these studies had undergone three steps of precipitation with isopropanol and dialysis against distilled water, 0.1 M NaCl and then distilled water once again. Samples were then digested with nucleases and a protease to remove any contaminating nucleic acid and protein. The lack of these contaminants was confirmed by UV spectroscopy. Finally, any contaminating lipopolysaccharides were removed by ultracentrifugation (100,000 x g, 4 h). Gel formation was tested for several of the EPSs *(P. flavescens* EPS, the *P. marginalis* type A, B, and C EPSs, and the *P. stutzeri* EPS) when applied in sugar-acid and calcium-type jellies according to standard A.O.A.C. procedures (49). For the sugar-acid type jelly test, total soluble solids were above 60% with a pH below 3.8 after boiling the polysaccharide (0.41% w/v) solution. For the calcium-type jelly test, calcium orthophosphate was added to the polysaccharide solution (0.08% w/v) to give 0.01% (w/v) at a total soluble solids level below 30% at room temperature. Bloom strength was quantified with a TA-XT2 Analyzer (Stable Micro Systems, U.K.) adjusted to 4 mm depression/second using a 0.5 inch plunger. Results indicated that none of the EPS samples tested performed as well as pectin in these tests (Table I).

Table I. Jelly Strength of *Pseudomonas* Exopolysaccharides When Applied in Sugar-Acid and Ca-Type Jellies

Polysaccharide Type or Source	*Sugar-Acid Jelly(g)*	*Calcium-Jelly(g)*
Pectin	150[a]	140[a]
P. flavescens EPS	24.7 ± 0.2[b]	3.7 ± 0.1
P. marginalis Type A	33.5 ± 0.1	3.5 ± 0.2
P. marginalis Type B	45.1 ± 0.2	4.1 ± 0.1
P. marginalis Type C	38.5 ± 0.5	6.8 ± 0.1
P. stutzeri ATCC17588	21.0 ± 0.3	3.5 ± 0.1

[a]IFT values for a pectin graded as standard.
[b]Values are averages of three replicate trials ± standard deviation.

The ability to form gels in the presence of calcium ions and after heating was determined with samples prepared at 5.0 mg/ml (for calcium-mediated gellation) or 10 and 20 mg/ml in Milli-Q purified water. To test for calcium-mediated gellation, the samples were dialyzed against 100 mM $CaCl_2$ with a commercial sample of algal alginate as a positive control. After overnight dialysis, only the algal alginate exhibited gel formation. For heat- mediated gelation, all samples were placed in a constant temperature water bath held at 95 C for 30 minutes. After gradual cooling

to room temperature only the commercial sample of curdlan, which was included as a positive control, gelled.

The weight average molar mass (Mw), Z average root mean square radius (Rgz) and the weight average intrinsic viscocity (IVw) of selected EPS samples were determined (Table II). Measurements were made by high-performance size

Table II. Physical Properties of *Pseudomonas* Exopolysaccharides

Polysaccharide Type or Source	*Mw ($x10^{-6}$)*	*Rgz (nm)*	*IVw (dL/g)*
P. fluorescens H13	2.85 ± 0.20[a]	123 ± 5	12.6 ± 0.3
P. "gingeri" Pf9	1.06 ± 0.04	108 ± 6	30.6
P. marginalis Type A	1.68 ± 0.03	108 ± 6	8.1 ± 0.1
P. marginalis Type B	1.02 ± 0.03	97 ± 7	35.3 ± 6.0
P. marginalis Type C	1.49 ± 0.03	112 ± 3	25.5 ± 1.0

[a]Values are averages of three replicate trials ± standard deviation except for *P.* "gingeri" Pf9 EPS (1 trial only).

exclusion chromatography with appropriate detectors. Freeze-dried samples were dissolved in 0.05 M $NaNO_3$, passed through a 0.2 µm Nucleopore filter (Costar Corp., Cambridge, MA) and equilibrated at 45 C for 20 minutes. Sample injection volume was 100 µl, and the mobile phase was 0.05 M $NaNO_3$. The mobile phase was degassed and filtered off line by passing it through a 0.4 µm Nucleopore filter. Detection of concentration was by differential refractive index.

For the Mw and Rgz measurements, solutions were pumped through three serially placed chromatography columns (all 8 mm i.d., 300 mm long): Shodex OH-pak SB-806 SB-805, and SB-803 (JM Science Inc., Buffalo, NY). The exclusion limits for these columns as specified by the manufacturer for pullulans were 4×10^7, $2 \times 10^6$and 1×10^5 g/mole, respectively. The Mw and Rgz were measured with a Dawn F MALLS photometer fitted with a helium-neon laser
(A= 632.8 nm) and a K-5 flow cell (Wyatt Technology, Santa Barbara, CA). The nominal flow rate was 0.7 mL/min and the sample concentration was 2 mg/mL.

For IVw measurements, solutions were pumped through four serially placed gel permeation chromatography columns (all 4.6 mm i.d., 250 mm long): two Synchropak GPC 4000 columns and one each of Synchropak GPC 1000 and GPC 100 (SynChrome, Inc., Lafayette, IN). The fractionation range for this column set approximates that of the Shodex columns used in the measurement of Mw and Rgz. The IVw was measured with a model 100 differential viscometer (Viskotek Co., Houston, TX) using a nominal flow rate of 0.45 mL/min and a sample concentration of 1 mg/mL.

The weight average molar masses of the exopolysaccharides in Table II ranged from 1.02 to 2.85×10^6, the Z average radii from about 97 to 123 nm and the weight

average intrinsic viscosities from about 8.1 to 35.3 dL/g. By way of comparison, a xanthan gum with a Mw of 1 x 10^6 has an IVw of about 24 dL/g in 0.5 M NaCl (50). From this comparison, one may conclude that the chain conformation of *P. marginalis* B and C, and *P.* "gingeri" EPS's may be comparable in extension to that of xanthan. To be sure of this, the physical properties of these three EPS's need to be measured at 0.5 M ionic strength rather than 0.05 as used in our study. The IVw's of EPS's from *P. marginalis* (type A) and *P. fluorescens* appear to be in about the same range as carboxymethyl or hydroxyethel cellulose, but higher than pectin, amylose, hydroxyethyl starch, pullulan, guar gum, and locust bean gum.

Conclusions

The search for new, industrially useful biopolymers obtained by microbial fermentation of renewable agricultural resources such as corn byproducts continues in earnest. The potential for the development of products with unique rheological properties as well as the biodegradable nature of bacterial polymers motivate such research. Although limited at present, a major market for biodegradable plastics made from bacterial polyalkanoates may develop. However, this will be dependent on the economics of the fermentation and manufacturing processes as well as the regulatory climate in the U.S.A.

Our studies with the group I pseudomonads, which comprise a rather limited number of bacterial species, demonstrated the unlimited potential for the discovery of novel biopolymers. Undoubtably, thousands of additional bacterial EPSs and other microbial polymers remain to be discovered, many of which will have unique properties for industrial applications. Studies of biopolymers produced by microbes found in extreme environments may be particularly fruitful.

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